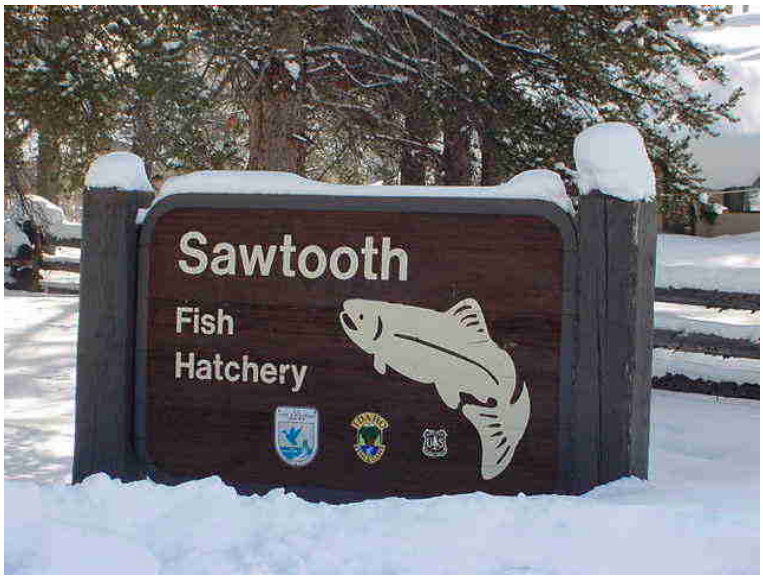
FISHERY RESEARCH

Sawtooth Settling Pond
Myxobolus cerebralis Exposure Trials



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ABSTRACT

Myxobolus cerebralis, the etiologic agent of whirling disease in salmonids, was first detected at Sawtooth Fish Hatchery in 1987. This facility uses river water from the Salmon River to culture Chinook salmon *Oncorhynchus tshawytscha* for the later portion of the 18 months required to attain smolt stage. Settling ponds at hatcheries with *M. cerebralis* positive water supplies are implicated in increasing parasitic loads in natural salmonids downstream of the hatchery effluent. The exact role of settling ponds in the spread, maintenance, and amplification of *M. cerebralis* in salmonid populations is unknown.

Ten-day exposure trials were conducted during September 2002 and April 2003, during peak *M. cerebralis* activity, to determine prevalence and intensity of *M. cerebralis* at the settling pond of Sawtooth Fish Hatchery. Temperature recording units were placed on each live-box to monitor water temperatures. Statistical analysis was used to determine significant differences in infection intensity and prevalence of infection among exposure sites within a particular exposure trial.

The September 2002 exposure trial detected significantly higher intensity of infection and prevalence of infection of *M. cerebralis* at the settling pond effluent site, when compared to the settling pond intake site. This suggested that the settling pond was producing elevated numbers of *M. cerebralis* triactinomyxons, the infective stage of this parasite. Although the settling pond effluent site had higher spore counts than the head-box of raceway 4 site, statistically this difference was insignificant. *Myxobolus cerebralis* spores were below levels of detection in sentinel fish exposed at the settling pond effluent site in the April 2003 exposure trial. *Myxobolus cerebralis* was detected at the other exposure sites during this trial, suggesting that triactinomyxon production had not yet commenced in the settling pond.

The findings of this research confirmed the ability of the settling pond of Sawtooth Fish Hatchery to produce *Myxobolus cerebralis* triactinomyxons. Furthermore, the settling pond of Sawtooth Fish Hatchery does not appear to significantly increase the *Myxobolus cerebralis* infectivity potential for the Salmon River. A management strategies that we recommend to further reduce the impacts of this parasite is to depopulate the settling pond of any fish, thus interrupting the life cycle of the parasite in the settling pond.

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INTRODUCTION

The earthen ponds of salmonid hatcheries located in whirling disease positive drainages have been suspected as being point sources for the introduction of the infective triactinomyxon (TAM) stage of *Myxobolus cerebralis* (MC) (Nehring and Thompson 2003). Earthen settling ponds should provide excellent habitat for the oligochaete host of MC, *Tubifex tubifex,* because of the reduced water current and high organic effluent from the hatchery (Kerans and Zale 2002). The potential TAM production from these settling ponds could be high and have a deleterious impact on wild salmonid populations below the hatchery effluent.

In 1987, both Sawtooth Fish Hatchery (SFH) and Pahsimeroi Hatchery (PH) were found to be whirling disease (WD) positive (IDFG database 2004). Both facilities utilize surface water from WD positive rivers, Salmon River for SFH and the Pahsimeroi River for PH, to culture Chinook salmon *Oncorhynchus tshawytscha* during the later portion of the 18 month hatchery rearing protocol. Idaho Department of Fish and Game (IDFG) initiated sentinel exposure trials at SFH and PH in 2000-2001 to demonstrate the relative exposure intensity of the two infective water supplies to determine methods to reduce exposure to the parasite and the need for expansion of well water capability for Chinook salmon culture at PH. Additionally the settling pond site at PH was demonstrated to have different monthly infection intensities relative to the intake site. This suggested that the PH settling pond could amplify the MC exposure to wild salmonids in the river below the hatchery.

The exact role of TAMs produced in settling ponds on WD in wild salmonids is unclear. The data gathered from this exposure trial will give insight into TAM production from the settling pond at SFH. Furthermore, the information obtained in these exposure trials will be utilized to make management recommendations to the SFH staff to reduce TAM production in the settling pond. This is assuming that reducing TAM production from the SFH settling pond will reduce potential infection jeopardy to the salmonids of the upper Salmon River.

STUDY SITE

This study was conducted at SFH, which is located in Central Idaho (Figure 1) located 8 kilometers (5 miles) south of Stanley, Idaho (UTM 11, 669592E, 4890183N). River water from the Salmon River is used to culture Chinook salmon during the last nine months of smolt production. Three exposure sites were selected at SFH (Figure 2) the first exposure site was in the head-box of small raceway four, the second exposure site was at the settling pond intake, and the third site was at the settling pond effluent (Figure 3 and 4). The head-box of raceway 4 represents the MC infectivity potential of the Salmon River water source. The settling pond intake site will demonstrate any changes in infectivity potential after the Salmon River water has passed through the raceways of the hatchery. The settling pond effluent site should detect the contributions of the settling pond to MC infectivity.

OBJECTIVES

We initiated this study to determine the relative infection prevalence and intensity of exposure to MC after a 10-day exposure in SFH settling pond during September 2002 and April 2003. In addition, recommendations will be made to the SFH staff in daily operations and hatchery renovation that would reduce the TAM production from the SFH settling pond.

METHODS

Hayspur and Troutlodge strain rainbow trout *Oncorhynchus mykiss* were reared from eggs at the Eagle Fish Health Laboratory (EFHL) in well water. Each exposure trial consisted of 20 rainbow trout placed in a cylindrical aluminum box (experimental unit) that measured 47 cm in length x 30.3 cm in diameter. Holding space in the EFHL wet lab restricted the number of experimental units to one per exposure site. Each experimental unit was equipped with a STOWAWAY XTI temperature logger to monitor water temperatures. The first exposure trial utilized Hayspur strain of rainbow trout *Oncorhynchus mykiss* averaging 1.6 g per fish. Troutlodge rainbow trout, averaging 0.5 g per fish, were used in the second exposure trial. Sentinel fish were exposed for 10 days during September 2002 and April of 2003 (Munson & Johnson 2003). The flow rate at small raceway 4 was at 300 gpm for both exposure trials, while the flow rate for the settling pond was at 28.87 mgd for the first trial and 21.65 mgd for the second exposure trial. After exposure, sentinel fish were returned to the EFHL wet lab and were held in 13°C well water until at least 1,300 Celsius temperature units (CTUs) were accumulated (approximately 100 days).

Following the holding period, the fish were euthanized with tricaine methane sulphonate (MS 222) and decapitated. Spores were enumerated from individual half-heads using a modification of the pepsin/trypsin digest method (Markiw & Wolf 1974; Burton et al. 2000; Munson & Johnson 2003). Estimated spore counts were used to compare relative exposure at the three exposure sites.

A Wilcoxon's Ranked Sum Test (WRST) α = 0.05 (Ott, 1977), was used to test for significant differences in spore count data between the exposure sites within a particular trial period. SYSTAT 10 (SYSTAT 2000) was used to analyze prevalence data within a trial period by performing Fischer's Exact Test (FET) α = 0.05.

RESULTS

Results from the first exposure trial conducted in September of 2002 demonstrated that an infection of MC could be acquired at all three exposure sites at SFH (Table 1). Two of the three temperature recorders that were attached to each experimental unit during this exposure trial were not launched correctly. The temperature logger that was launched correctly was attached to the experimental unit stationed in the head-box of raceway 4. The maximum temperature recorded during the exposure period was 16.03°C and the minimum temperature was recorded at 7.63°C. The average water temperature during the first exposure trial was 11.61°C. *Myxobolus cerebralis* was detected at a 72.4% prevalence level (21/29 fish positive for MC) at the exposure site located at the head-box of raceway 4 and averaged 4,886 spores

per head (range 0-16,700). The sentinel fish from the settling pond intake site had a MC prevalence of 8.7% (2/23 fish MC positive) with an average of 148 spores per head (range 0-1,667). Samples from the settling pond effluent site had a prevalence of 92.0% (23/25 fish MC positive), and averaged 10,136 spores per head (range 0 - 60,000).

We failed to detect significant differences in the intensity of infection of MC (WRST, ($P \geq 0.05$) and prevalence of infection of MC (FET, P = 0.0860686) between raceway 4 and settling pond effluent exposure sites in the September 2002 trial. Significant differences were detected in prevalence (FET, P = 0.000003795) and infection intensity (WRST, $P < 0.05$) when the head-box exposure site of raceway 4 was tested against the settling pond intake exposure site. Significant differences were also detected when the settling pond effluent site was tested against the settling pond intake site (FET, P = 0.0000000004) during the exposure trial conducted in September 2002 (Table 2).

Results from the second exposure trial conducted in April of 2003 demonstrated MC infections in sentinel fish placed at the head-box of raceway 4 and at the settling pond intake site (Table 1). The sentinel fish of head-box of raceway 4 site had a prevalence of 35% (7/20 fish MC positive) and 4,400 spores per head were counted (range 0 - 33,333). The average water temperature for the head-box exposure site during this trial was 5.17°C (range 0.97°C to 10.96°C). The sentinel fish of settling pond intake site had a prevalence of 20% and 830 spores per head (range 0 - 6,667). The average temperature for the settling pond intake site during this exposure trial was 4.99°C (range 1.19°C to 10.26°C). At the settling pond effluent site, MC was not detected in the sentinel fish. The average temperature was 4.98°C (range 2.94°C to 7.79°C).

We failed to detect significant differences in the intensity of infection of MC (WRST, $P \geq 0.05$) and prevalence of infection of MC (FET, P = 0.480115354) between the raceway 4 and settling pond intake sites in the April 2003 exposure trial. Significant differences were detected in infection intensity (WRST, $P < 0.05$) and prevalence of intensity (FET, P = 0.0003342575) between the raceway 4 and settling pond effluent sites and between the settling pond intake and settling pond effluent sites (WRST, $P < 0.05$) (FET, P = 013191211) conducted in April 2003 (Table 2).

DISCUSSION

In the September 2002 exposure trial, intensity and prevalence at the raceway 4 and settling pond effluent exposure sites were similar (Table 2). Samples from the settling pond effluent site had a higher prevalence, higher mean estimated spore per head, and a larger spore count range than either of the other sites (Table 1). Although the sentinel fish from the settling pond effluent site demonstrated a higher prevalence and spore count, these differences when tested with FET and WRST ($\alpha = 0.05$) were insignificant. This suggests the discharge of SFH settling pond effluent does not amplify *M. cerebralis* in the Salmon River as expected. Other earthen pond studies at hatcheries in MC positive locations, such as in Colorado Department of Wildlife (CDOW) facilities, had very high MC activity until management options to lessen MC fish were applied (Nehring and Thompson 2003). Samples from the settling pond intake site, which is located between the raceway 4 site and the settling pond effluent site, detected significantly lower levels of intensity of infection and prevalence of infection. This demonstrates the ability of the fish cultured in the hatchery's raceways to filter the MC TAMs from the water

before entering the SFH settling pond. The increases in prevalence and MC spore detection from the samples from the settling pond intake site to the settling pond effluent site, demonstrates the settling pond's ability to produce MC TAMs. Prior September exposure trials in the head-box of raceway 4 (Munson and Johnson 2003) found higher average spore counts per head (approximately 5,150 spores per head) than in this exposure trial (approximately 4886 spores per head). In the 2000 exposure trial (Munson and Johnson 2003) found a prevalence of infection of 85% as compared to this exposure trial with a prevalence of infection of 72.4%. The average water temperature during the exposure trial in 2000 was 13°C, while the average water temperature during the exposure trial in August 2002 was 11.61°C. This may account for the differences in MC infectivity.

When prevalence and infectivity is compared between the September 2002 exposure trial at the effluent of SFH settling pond and the effluent of Pahsimeroi Hatchery in the 2000 (Munson and Johnson 2003), both settling ponds had similar average temperatures during the trials (11.61°C at SFH in 2002, and 12.0°C at PH in 2000) which may explain similarities in results. Prevalence of infection was measured at 80% at the PH settling pond effluent site in 2000, with a mean spore per head count of 9,350. Similarly, SFH had a prevalence of 92%, and 10,136 mean spores per head. This demonstrates that the SFH settling pond has the ability to produce TAMs at a similar rate as the PH settling pond during warmer water temperatures.

In the April 2003 exposure trial, raceway 4 and settling pond intake exposure sites were statistically similar in infection intensity and prevalence of infection (Table 2). Raceway 4-exposure site had an average spore count per head of 4,400 during the April exposure trial. Prior exposure trials conducted during April 2000 (Munson and Johnson 2003) and April 2001 (Cavender et al. 2003) found higher average spore counts at the same location. Munson and Johnson (2003) found an average spore count per head of approximately 15,000, with a prevalence of infection of 20%, while Cavender et al. (2003) found an average spore count per head of 8,250 and a prevalence of infection of 65%. *Myxobolus cerebralis* spores were not detected at the settling pond effluent site during this exposure trial. Seasonal fluctuations of infectivity have been established in western rivers and hatcheries utilizing MC positive river water sources (Munson and Johnson 2003; Vincent 1998; Lukins et al. 2003, Burton et al. 2000). Possibly the MC TAM production had not started in the settling pond prior and during this exposure trial. Further exposure trials during this time-period may be warranted.

When the data from the settling pond effluent site of the second exposure trial are compared to the PH settling pond site in 2000 (Munson and Johnson 2003), the SFH settling pond in April 2003 had an average temperature during the exposure trial of 4.98°C. *Myxobolus cerebralis* was not detected in any of the sentinel fish. In the PH settling pond during the exposure trial of 2000, the average temperature during the exposure trials was 9.3°C. During this trial, the prevalence of infection was 89%, with a mean spore per head count of 14,670. The difference most likely is due to the temperature differences in the two trials. Lower temperatures experienced at SFH throughout the year should reduce overall TAM production in the settling pond, as compared to PH, except when temperatures are within the optimum range for TAM production. Thus, SFH should not present as great of risk in transmitting MC to natural salmonid populations below the hatchery effluent.

The need to alter SFH or the fish production programs was not established because the spore levels during these exposure trials were low compared to other studies (Nehring and Thompson 2003). Best management practices (BMP) are being implemented at hatcheries located in MC positive locations. These BMP for hatcheries were outlined by the Colorado

Division of Wildlife (CDOW) (Kreiger et al. 1998; Nehring and Thompson 2003) and were used to reduce TAM levels in CDOW fish hatcheries. We recommend similar actions that would help interrupt the life cycle of MC at IDFG fish hatcheries when necessary. Settling pond renovation or annual cleaning and drying are impractical since fish production programs overlap, and SFH is in constant use. The IDFG hatcheries already include daily removal of fish mortalities and off-site disposal as well as not stocking settling ponds for public angling as standard operational procedures. The SFH staff should depopulate any fish populations from the settling pond, thus interrupting the life cycle of the parasite within the settling pond.

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Table 1. Exposure Trial Data From Sawtooth Settling Pond Exposure Trials.

Location	Average Temp*C (Range:)	Positive Sentinels/Total	Prevalence (%)	Mean Estimated Spores/head (Range:)
Trial 1. (Sept. 6-16, 2002):	**Hayspur RBT Mean Weight at Exposure 1.6g/fish**			
Raceway 4	11.61(7.63-16.03)	21/29	72.4	4886 (0-16,700)
Settling Pond Intake	NR	2/23	8.7	148 (0-1,667)
Settling Pond Effluent	NR	23/25	92	10,136 (0-60,000)
Trial 2. (April 1-10,2003):	**Troutlodge RBT Mean Weight at Exposure 0.5g/fish**			
Raceway 4	5.17(0.97-10.96)	7/20	35	4,400 (0-33,300)
Settling Pond Intake	4.99(1.19-10.26)	4/20	20	830 (0-6,667)
Settling Pond Effluent	4.98(2.94-7.79)	0/36	0	0

NR = Not Recorded. Temperature recorders not launched correctly.

Table 2. Statistical Comparisons of the Sawtooth Settling Pond Exposure Trials.

Site Comparison	Wilcoxon Ranked Z Value	Sum Test t-test P	Prevalence Fisher's Exact Test P
Trial 1			
RW 4 v SPI	4.68	P<0.001	0.000003795
RW 4 v SPE	0.87	P>0.05	0.0860686
SPI v SPE	5.57	P<0.001	0.000000004
Trial 2			
RW 4 v SPI	1.65	P>0.05	0.480115354
RW 4 v SPE	3.75	P<0.001	0.000334257
SPI v SPE	2.76	P<0.010	0.013191211

Figure 1. Location of Fish Hatcheries in Idaho.

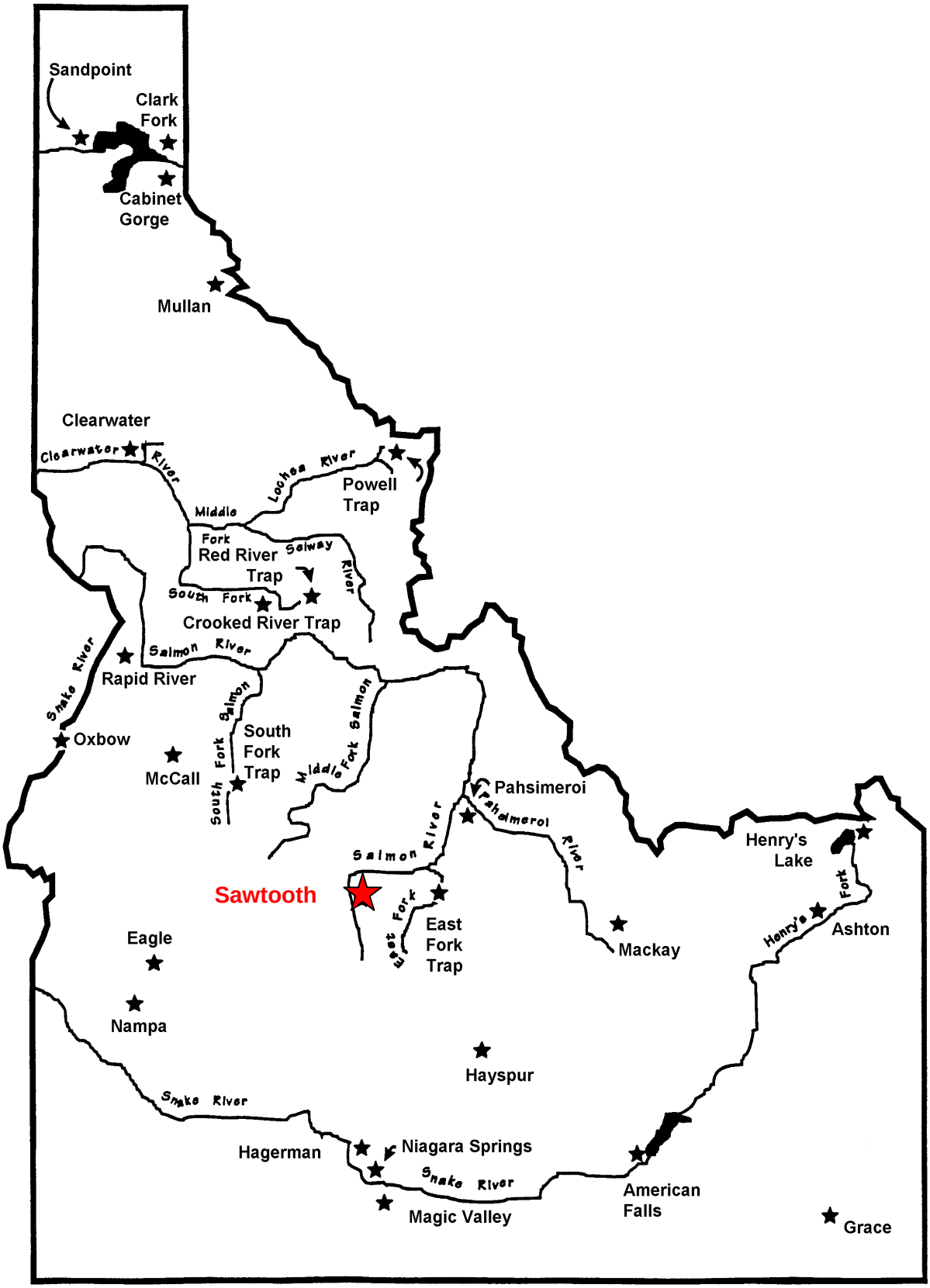

Figure 2. Sawtooth Fish Hatchery

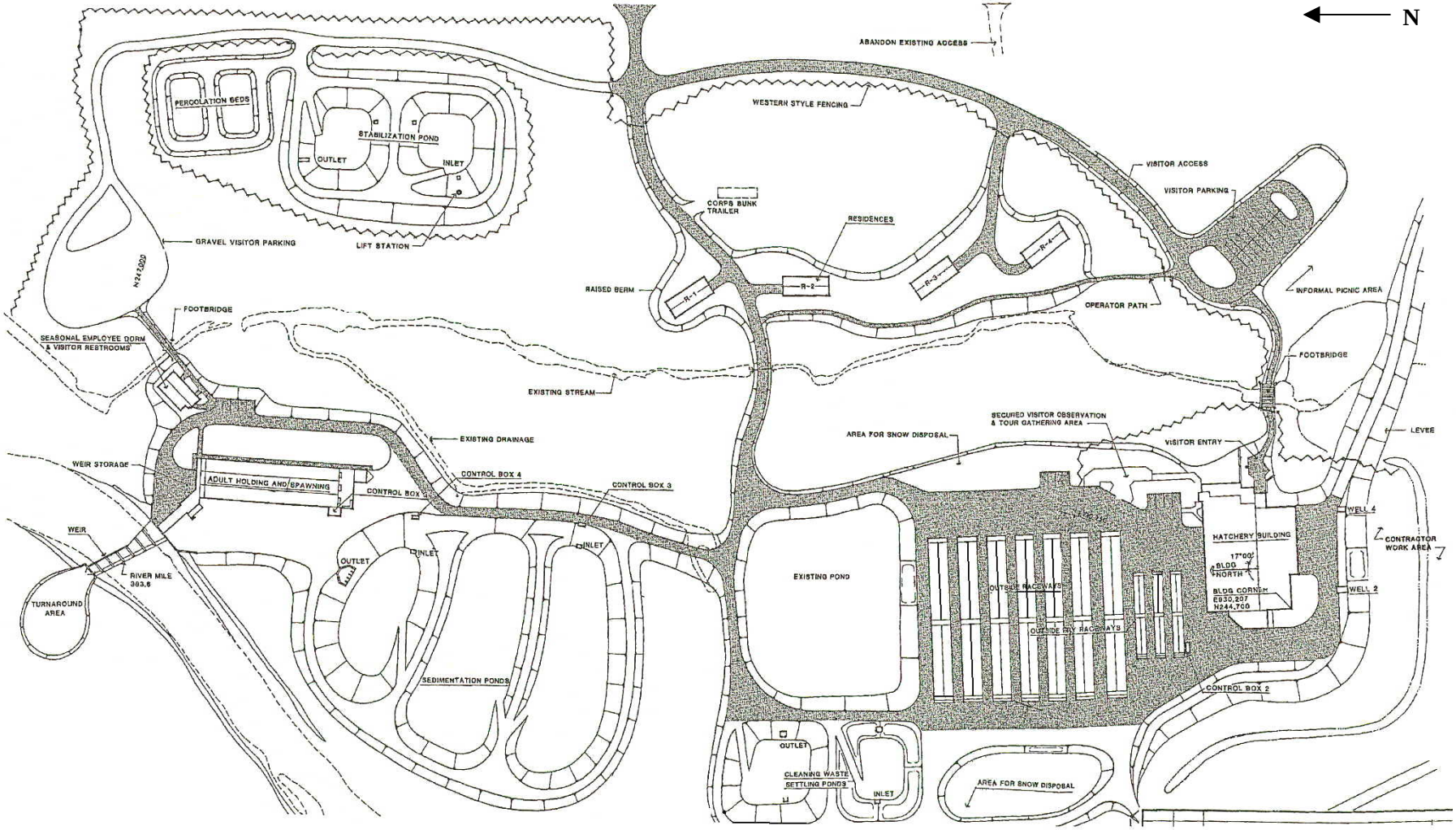

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Figure 3. Raceway 4 Exposure Site

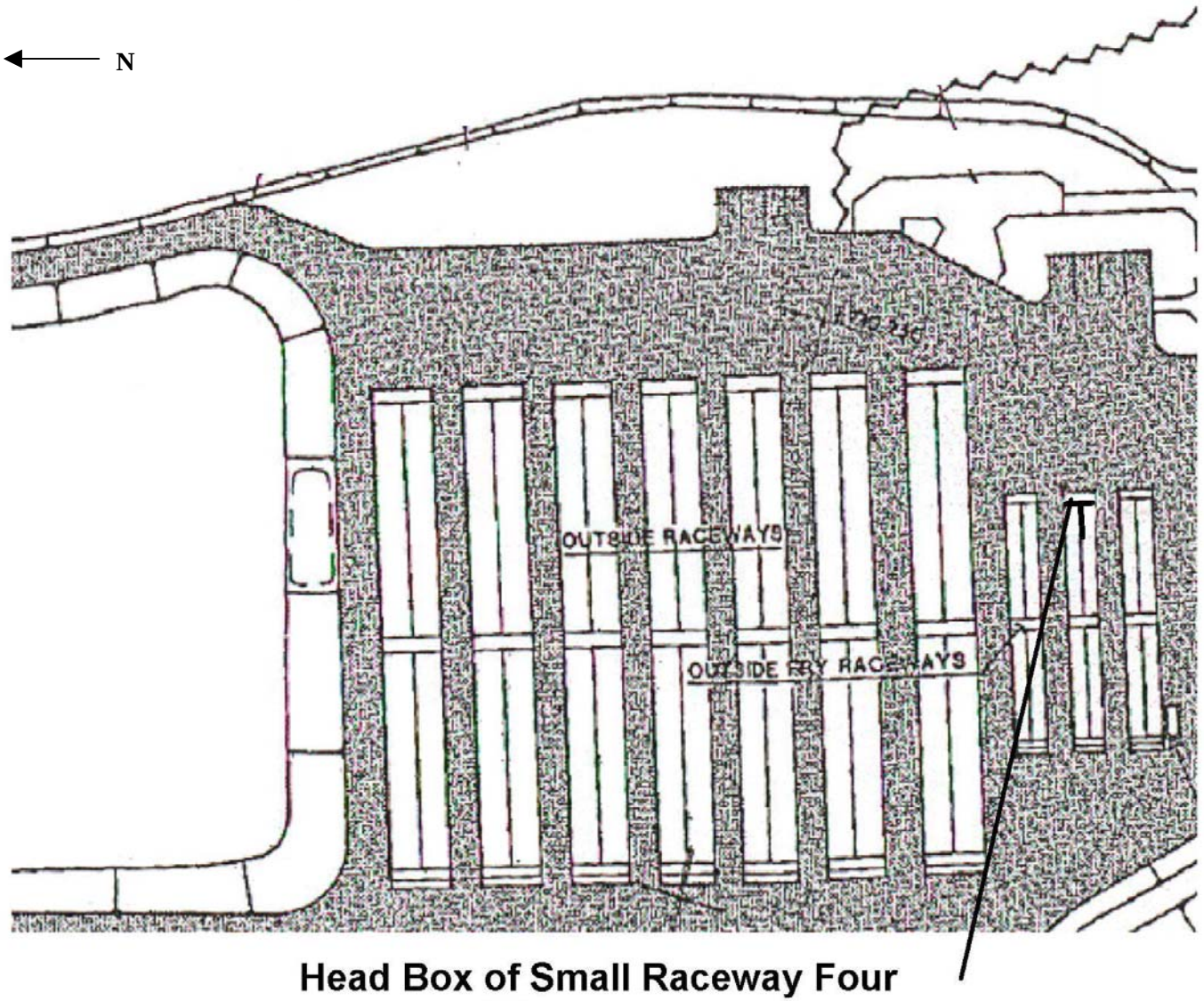

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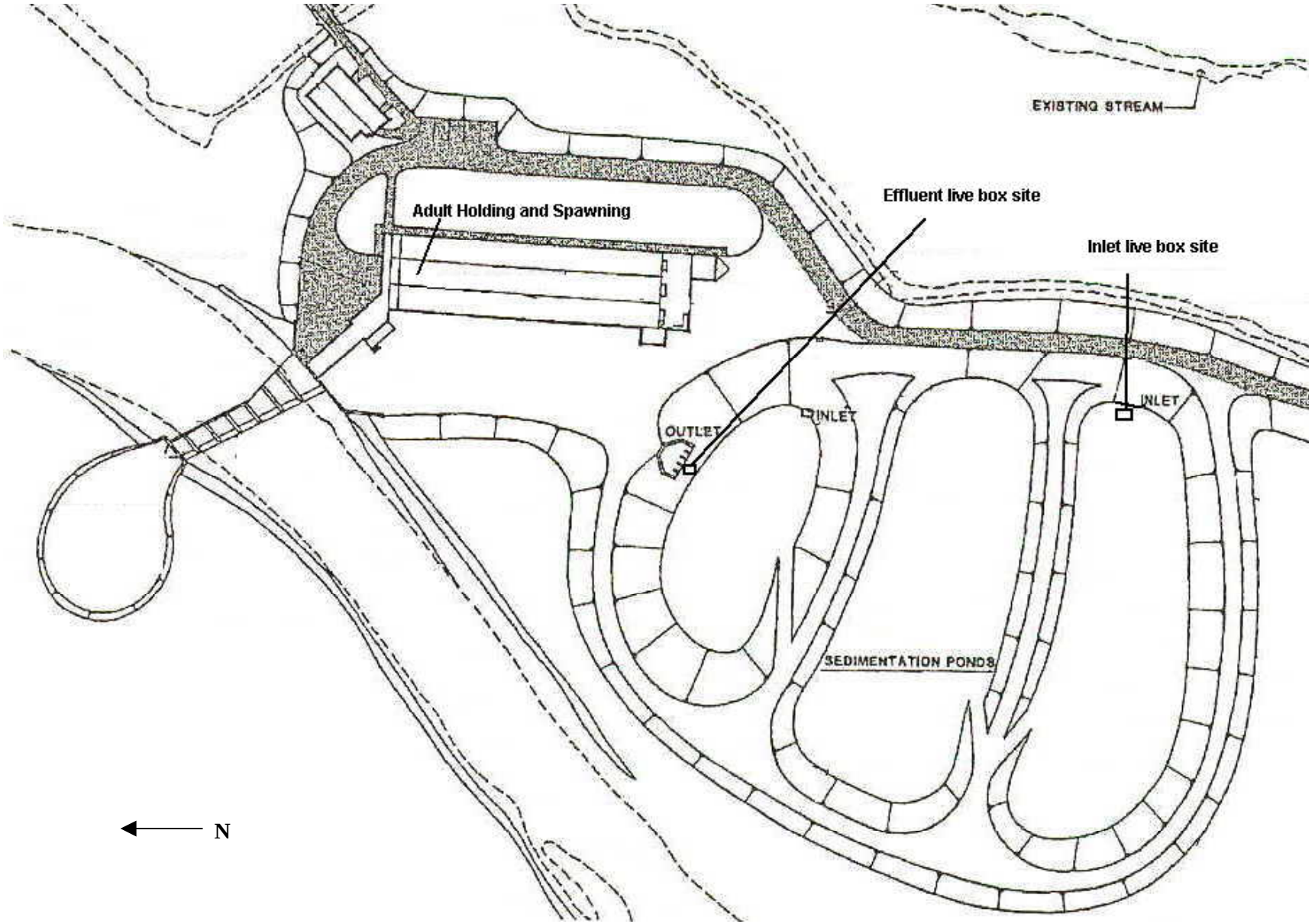
Figure 4. Sawtooth Settling Pond



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